reservoirs for BLM experimentation; the Teflon film presents a micrometer-size aperture, located at the intersection of the microchannels, and across which BLMs are made. Leakage-free assembly of the three layers is demonstrated using an optical adhesive.

The closed configuration of the device prompted us to develop a novel methodology for BLM preparation. Lipid solution and buffer are successively flushed in both channels, so that the lipid plug deposited in the aperture spontaneously thins into a bilayer. BLM formation is monitored electrically (patch-amplifier) and optically ((fluorescence) microscopy). This lipid-plug-thinning technique gives highly stable BLMs (> 7 hrs lifetime) with an almost 100% success yield. The resulting membranes exhibit reproducible characteristics in terms of sealing quality (14 ± 4 giga-ohm) and surface area (~80% aperture coverage, measured optically and electrically (10 ± 3.4 pF capacitance)). Insertion of alpha-hemolysin (in the BLM) confirms the formation of a bilayer structure and demonstrates the potential of the platform for single protein measurements.

We will present the detailed fabrication of the microfluidic platform, BLM formation and characterization in the closed environment, and on-chip single proteins studies using optical and electrical techniques.

2838-Plat

Interfacial Tension Controlled Fusion of Individual Femtoliter Droplets and Triggering of Confined Chemical Reactions on Demand

Charles P. Collier, Scott T. Retterer, Seung-Yong Jung.
Oak Ridge National Laboratory, Oak Ridge, TN, USA.

Droplet-based microfluidic platforms offer many opportunities to confine chemical and biochemical reactants in discrete ultrasmall reaction volumes, and investigate the effects of increased confinement on reaction dynamics. Current state-of-the-art microfluidic sampling strategies for creating ultrasmall reaction volumes are predominately steady-state approaches, which result in difficulty in trapping reacting species with a well-defined time-zero for initiation of biochemical reactions in the confined space. This talk describes stepwise, on-demand generation and fusion of femtoliter aqueous droplets based on interfacial tension. Sub-millisecond reaction times from droplet fusion were demonstrated, as well as a reversible chemical toggle switch based on alternating fusion of droplets containing acidic or basic solution, monitored with the pH-dependent emission of fluorescein.

2839-Plat

Elucidation and Control of the Hybridization Chain Reaction

Victor A. Beck, Justin S. Bois, Robert M. Dirks, Niles A. Pierce.
Caltech, Pasadena, CA, USA.

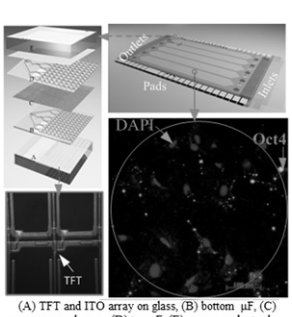
We previously introduced hybridization chain reactions (HCR), in which metastable DNA hairpins undergo conditional self-assembly to form long nicked double-stranded 'polymers' in the presence of a DNA initiator molecule (RM Dirks and NA Pierce, PNAS 2004, 10, 15275). HCR systems have been engineered to function as orthogonal in situ amplifiers for multiplexed bioimaging (HMT Choi et al., Nat Biotech, in press) and as programmable mechanical transducers for selectively killing cultured human cancer cells (S Venkataraman et al., PNAS 2010, 107, 16777). Here, we model the equilibrium and kinetic properties of HCR, revealing sources of non-ideal behavior and methods for controlling system performance. Our results demonstrate that HCR is accurately modeled as a living alternating copolymerization.

2840-Plat

Precise Transfection Control of Cell Reprogramming Factors via a High Throughput Electroporation System

Ebrahim Ghafar-Zadeh, Erh-Chia Yeh, Chi-cheng Fu, Luke P. Lee.
University of California, Berkeley, Berkeley, CA, USA.

There is great interest in reprogramming of human fibroblasts to induced pluripotent stem (iPS) cells as an autologous cell source for therapeutic applications, but the utility of iPS Cells is hampered by the use of viral delivery systems. We demonstrate the ability to reprogram the cells by transducing the transcription factors including Oct4 via electroporation method (EPM). A major drawback of conventional EPMs is high cell mortality and low efficiency. To overcome these limitations, we are using a novel hybrid microfluidic/thin film transistor system (μF-TFTS) to precisely control the electroporation potential for each individual cell. Here, we present the recent works on the characterization of μF-TFTS for electro-transfecion of cell reprogramming factors. We search for optimum electrical with accuracy of 0.003% over dynamic range to transfect a certain cell programming factor into single cells using μF-TFTS. We perform the electroporation on 60,000 sample five different factors (PI, DAPI, Oct4) in a few second. This technologically integrated approach offers new prospects for understanding the biophysical complexity of cell reprogramming as well as cell behaviors in a precisely controllable electrical field using a high throughput and rapid EMP system.



(A) TFT and ITO array on glass, (B) bottom μF, (C) porous membrane, (D) top μF, (E) common electrode.

2841-Plat

A pore-Cavity-Pore Device to Trap and Investigate Single Nano-Scale Objects in Femto-Liter Compartments: Confined Diffusion and Narrow Escape

Martin Langecker, Daniel Pedone, Robin D. Nagel, Friedrich Simmel, Ulrich Rant.
Technische Universität München, Garching, Germany.

Spatial confinement from the nano- to the micro-scale is ubiquitous in nature. Striving to emulate biological compartmentalization and to understand the fundamental physical behavior of molecules in confined domains, micro- and nano-structuring techniques have been used extensively to create artificial devices comprising liquid-filled compartments and channels. In addition, the development of robust solid-state structures, which allow for the observation and manipulation of single nano-scale objects is key for the realization of future lab-on-chip devices with improved functionality.

Here we introduce an electrically addressable nano-fluidic silicon device that consists of two stacked nanopores forming the in/outlets to a pyramidal cavity of micrometer dimensions, i.e. femto-liter volume. The electrical properties of the PCP structure are investigated by impedance spectroscopy. Furthermore, we present a FEM simulation of the electric field inside the device. We then demonstrate how individual fluorescent nano-particles and DNA can be actively (by electrical means) and passively (entropically driven) loaded into, trapped inside, and unloaded from the 'pore-cavity-pore' (PCP) device.

A fundamentally important problem in biology is the escape of nano-objects from a micro-domain through a small opening (narrow escape problem). Using the PCP device it is possible for the first time to obtain data on the narrow escape time under well-defined geometrical and experimental conditions. Single particle tracking and residence time data are presented and quantitatively compared to random walk simulations and analytical theories on confined diffusion and the narrow escape problem. Furthermore, we extend the escape studies towards polymeric analytes like DNA.

Symposium 20: Single Molecule Biophysics of the Central Dogma

2842-Symp

Single Molecule Probing of Helicase Dynamics

Sua Myong.
Univ of Illinois, Urbana - Champaign, Urbana, IL, USA.

Helicase is an important genome caretaker protein which plays a central role in biological processes including replication, transcription, recombination and repair of DNA. Helicase, by definition is an enzyme which unwinds a duplex nucleic acid into its single strand components. However, many helicases found in biological systems are not processive unwindases. We have employed single molecule fluorescence detection techniques to unveil detailed mechanism of noncanonical helicases which perform unique translocation activities.

Using single molecule FRET, we discovered an unexpected shuttling motion of an e. coli protein, Rep. The ATP-driven repetitive translocation of Rep on single stranded DNA was visualized as a sawtooth pattern of FRET change. Furthermore, we showed that the repetitive motion may play a functional role of maintaining single strand DNA free of unwanted protein such as recombination protein, RecA (1).

We have developed a newly developed single molecule fluorescence method, termed "protein induced fluorescence enhancement" (PIFE) to probe a translocation activity of an antiviral receptor protein, RIG-I. The translocation was inhibited by its own N-terminal CARD domain whereas the inhibition was completely lifted by a viral mimic RNA which contained unique pathogenic signatures. Our result demonstrates how RIG-I exhibits an exquisite mechanism to self-regulate its activity by sensing the pathogenic moiety (2).

Reference

1. S. Myong, I. Rasnik, C. Joo, T. M. Lohman, T. Ha, Nature 437, 1321 (Oct 27, 2005).
2. S. Myong et al., Science 323, 1070 (Feb 20, 2009).